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An Integrated Research Program on Viral Hemorrhagic Septicemia in the Great Lakes

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Background: Viral hemorrhagic septicemia (VHS) is considered to be the most important viral disease of finfish worldwide and is listed as a reportable disease by many nations and international organizations. Prior to 1988, the causative agent, a rhabdovirus (VHSV), was not known to occur outside continental Europe where it remains a major pathogen affecting rainbow trout aquaculture. Subsequently, a North American strain of VHSV (known as Genotype IV) was found to be widespread among marine fish on the Pacific coast of North America where it has been shown to be highly pathogenic for marine species, especially herring. Surveys of marine fish in other regions of the world have revealed that VHSV is also common among marine species in the North Atlantic, the Baltic Sea, the North Sea and Japan. In 2005-2006, reports from the Great Lakes region indicated that wild fish had experienced disease or, in some cases, very large die-offs from a related strain of VHSV now identified as Genotype IVb. As of April 2009, VHSV has been isolated from various species of fish in much of the Great Lakes Basin including Lake Huron, Lake Michigan, Lake St. Clair, Lake Erie, Lake Ontario, the Niagara and St. Lawrence Rivers and from inland lakes in New York, Michigan and Wisconsin. The type IVb isolate found in the Great Lakes region is the only strain of VHSV that has been linked to large natural mortalities among freshwater species. In 2008, an isolate of VHSV was obtained from muskellunge broodstock collected from a reservoir in Ohio that drained into the Mississippi River. To date, significant disease or mortality has been reported in muskellunge, freshwater drum, goby, burbot, yellow perch, gizzard shad, and smallmouth bass and VHSV has been isolated from more than 20 additional species in the region, but the magnitude of any additional mortality has not been reported. The presence of a reportable pathogen in the region, the large-scale mortalities among wild species, the potential impacts on commercial aquaculture and the impending disruptions of interstate and international trade have caused substantial concern among many entities.

Research Needs: In 2007, a research plan was prepared with topics listed approximately in order of the immediacy of the need for information by federal and state management agencies or in the order in which the research might be staged to most effectively build upon results from prior work. Soon after, several agencies in the US and Canada began to fund VHSV research and surveillance activities. This 2009 update to the VHSV research plan is intended to help ensure priority needs are met, maximize the use of scarce resources and avoid duplication. In this revision, some topics have been removed that are essentially complete or appear not to be needed and new topics have been added that have emerged as relevant. In addition to new research, some of the needed information can be deduced from knowledge of other genotypes of VHSV (e.g. type I in Europe or IVa in Western North America) as well as of related fish rhabdoviruses (e.g. infectious hematopoietic necrosis virus in Western North America or hirame rhabdovirus in Japan). The sections below contain the revised strategic plan along with a narrative indicating at least some of the ongoing research and where information gaps remain.

1. Improve Surveillance to Define Host and Geographic Range and to Detect Spread

In addition to helping to establish the current host and geographic range of VHSV as it emerges in the Great Lakes Basin and spreads to nearby systems, this work provides important information for epidemiology and risk assessment.

- Throughout the Great Lakes Basin, Upper Mississippi Drainage and other high risk areas (e.g. watersheds with traffic in baitfish or other identified pathways of risk), maintain surveillance using samples from diseased and healthy fish or water samples to define the

present host and geographic range of VHSV, to detect spread of the virus into new hosts or geographic areas and to provide information that will help further define pathways of risk.
- Increase surveillance of marine and estuarine fish on Atlantic seaboard of US and Canada to identify natural reservoir species and possible origin of VHSV genotype IVb
- Establish better coordination for response to fish kills and their reporting
- Establish a shared database of surveillance activities.
- Investigate use of highly susceptible sentinel fish species for surveillance activities
- Use surveillance data to gain insights into reservoir species and prevalence of carriers

Surveillance activities by state, provincial, federal and university diagnostic laboratories have increased, resulting in important information about the range of susceptible species and geographic distribution of VHSV IVb. The data have also provided important insights into the epidemiology of the virus such as the apparent introduction and subsequent clearance of VHSV from fish in a lake where summer temperatures may have not been permissive for virus survival and the window of time and temperature at which VHSV infections of yellow perch occur in Lake Erie. Work has been conducted to determine optimal cell lines and incubation conditions for isolation of VHSV IVb. National reference laboratory services for VHSV are in place in the US and Canada.

2. Biosecurity for Aquaculture Facilities

To date, VHSV IVb has only been recovered from wild or free-ranging fish. The research in this section is needed to assist in preventing the movement of VHSV into commercial and public aquaculture facilities. In addition to disease inspections and movement restrictions, the effectiveness of normal disinfection procedures needs to be evaluated.

- Test disinfection methods for hatchery facilities and equipment - Chlorine, iodine
- Test egg disinfection methods for various cultured species - iodine
- Evaluate methods for disinfection of hatchery water supplies - UV irradiation, ozone
- Test effluent treatment methods for quarantine facilities and processing plants

Published literature suggests VHSV IVb should be easily inactivated by low levels of free chlorine or iodine, however, much of the literature fails to distinguish between free and total halogen concentrations or to control for pH or temperature. States and Provinces in the Great Lakes Basin have adopted egg disinfection protocols using iodophor. Work to demonstrate efficacy of iodine for disinfection of VHSV contaminated eggs is ongoing. Extension information on VHSV is being prepared for the industry. Tests of uv inactivation have been conducted.

3. Improved Diagnostic Methods

Isolation of VHSV in cell culture is the current method specified for both surveillance and disease-free certification examinations. Newer methods will reduce the time required for these assays; however, in order to be approved for use, the new methods need to be validated against a "gold standard" (currently cell culture) according to published approaches (e.g. OIE).

- Validate and compare new rapid molecular diagnostic assays (e.g. qPCR)
- Develop and validate methods for detecting and quantifying virus in large-scale water samples (e.g. lakes, rivers, ballast tanks or in areas of fish kills)
- Develop serological methods to detect antibodies to VHSV as an indicator of past exposure

In addition to published quantitative PCR assays for detection of other strains of VHSV, new qPCR assays have been developed at several laboratories and are in various stages of validation. A competitive ELISA for quantification of serum antibody is in development. Methods to concentrate and detect VHSV from water samples have been adapted and used in survey efforts.

4. Genetic Typing and Molecular Epidemiology

Sequence analysis of informative portions of virus genomes can provide insights about the epidemiology of virus diseases as well as about virus evolution, antigenic drift, recombination or other genetic changes that allow adaptation to new hosts or to higher virulence.

- Conduct genetic typing of VHSV isolates from the Great Lakes and elsewhere in North America. Interpret phylogenetic trees for epidemiological and evolutionary inferences and to identify genetic changes associated with changes in host range or virulence.
- Archive virus isolates and genetic sequence data
- Develop and maintain a searchable database accessible by internet of VHSV isolates and genetic typing information.

To date, a portion of the glycoprotein or nucleoprotein genes have been sequenced for many isolates of VHSV IVb from the Great Lakes region. All are remarkably similar (for a fish rhabdovirus) suggesting VHSV was recently introduced into the region. Databases of sequence data and information about the isolates are in final stages of completion.

5. Development of Laboratory Challenge Model(s)

Much of the research needed to understand the epidemiology of VHSV in the Great Lakes will require a laboratory challenge model using a fish species that is relevant to the region, easy to maintain, available as pathogen-free stock and at least moderately susceptible to VHSV IVb, preferably by immersion. Optimally, the challenge model would use a fish species that can be cultured in a controlled environment, obtained as at various ages and for which various genetic lineages are available. For each model species, the following steps are needed:

- Standardize culture protocols and conditions that can be used to produce and distribute healthy experimental stocks of fish to laboratories of various principle investigators.
- Determine standard conditions for virus challenge including: route of exposure (immersion, injection, and cohabitation), dose, age, temperature and duration of observation. The goal is to define conditions to reproducibly cause both low (20-30%) and high (60-80%) mortality
- Determine virus titer in tissues over duration of challenge and in fish that survive challenge
- Characterize pathology in model infection

Initial work has shown that cultured stocks of pathogen-free yellow perch are available and are susceptible to laboratory infection with VHSV IVb, producing high titers of virus in tissues and pathology similar to that observed in the wild. Other species (e.g. muskellunge, walleye and zebrafish) have also been shown to be susceptible in laboratory trials and should be useful.

6. Test Potential Host Range of VHSV IVb

In addition to increased surveillance to determine the existing host and geographic range of the Great Lakes VHSV strain IVb, it will be useful to determine the potential host range of the virus using controlled laboratory challenge studies of selected cold- and cool-water species. Optimally, these studies would include pathology and be conducted in conjunction with a laboratory model

that can be used as a positive control for each experimental virus challenge in order to better assess relative susceptibility. Alternatively, studies could use injection of a single high dose of virus, at a single temperature appropriate for the fish species. Fish would be monitored for disease signs, mortality, and virus titer to detect asymptomatic infections. Species should include:

- Wild fish predator and prey species important to resource management in the Great Lakes
- Aquaculture species relevant to the Great Lakes region
- Aquaculture species of national importance

Completed, ongoing and planned studies include susceptibility tests of rainbow trout, fathead minnow, yellow perch, Chinook salmon, Pacific herring, muskellunge, tiger muskellunge, lake trout, largemouth bass and lake sturgeon.

7. Epidemiology and Disease Ecology

The research in this section is designed to further our understanding of how the virus is maintained and transmitted in natural systems and to develop information that can be used to predict future effects upon wild species. In addition, this work will provide information about the risk to fish in aquaculture facilities from an endemic reservoir among free-ranging fish. This work will use both field studies and controlled laboratory research.

- Determine virus survival in water under different chemical and temperature conditions
- Investigate the existence, nature and duration of a carrier state as source of virus spread
- Identify virus vectors and/or reservoir species in the Great Lakes system
- Identify routes of virus traffic between watersheds (e.g. role of baitfish)
- Identify variation in susceptibility among different genetic stocks of a given fish species
- Determine timing, duration and magnitude of virus shedding from infected fish
- Determine factors controlling virus shedding
- Determine waterborne virus levels needed for transmission among susceptible species
- Determine the effect of fish density on transmission among susceptible species
- Investigate how the ratio of susceptible to immune fish affects transmission

Experimental trials have determined the stability of VHSV IVb at differing temperatures in several water types. Surveillance activities have extended knowledge of susceptible species that might serve a reservoirs or carriers. An expert panel was used to identify the strength of various pathways of VHSV introduction and spread. Unexpected infections of invertebrates and sea lampreys have been detected. Significant differences in mortality were observed for tiger muskellunge hybrids made from different parental stocks.

8. Effect of Temperature on VHSV Infections of Fish

Temperature is one of the most important environmental factors controlling the host-pathogen relationship in poikilotherms. Work in this section will define the effects of temperature on virus growth in cell culture and on the course of disease in laboratory model infections.

- Determine the effect of temperature on virus replication in cell culture
- Determine the effect of temperature on susceptibility to infection in fish
- Determine the effect of temperature on disease progression in fish
- Determine the effect of temperature on virus clearance in fish

Early research showed that VHSV IVb will not grow in cell cultures at 25°C, but the ability of fish to clear VHSV may occur at temperatures below this. Some ongoing work is being conducted on effect of temperature on pathology and clearance.

9. Effect Other Environmental and Physiological Conditions on VHSV Infections of Fish

Other environmental and physiological conditions are known to affect susceptibility to virus infections of fish. Work in this section will define the effects of stress and spawning on the course of disease in laboratory model infections.

- Determine the effects of contaminants on susceptibility to VHSV
- Determine the effects of stressors such as crowding and handling
- Determine effects of stressors on duration or reactivation of virus infection
- Determine the effect of fish size, age or reproductive status on susceptibility

10. Ability of VHSV IVb to Adapt to Other Hosts

This research will explore the ability of the Great Lakes strain of VHSV to adapt to new host species or to increase in virulence as was observed for the European strain of VHSV (Genotype I). Initially endemic in wild marine fish, the practice of feeding raw marine fish in freshwater aquaculture provided an opportunity, over time, for VHSV type Ia to become highly virulent for rainbow trout. A similar host jump was documented for the emergence of a novel genotype of another fish rhabdovirus, IHNV, which has become one of the most important viral pathogens of rainbow trout in the US. The results will be useful to assess the risk to new species including those important to aquaculture.

- Examine the ability of immune selection to drive virus evolution
- Test the ability of serial passage in a species to increase virulence in that species
- Test the ability of infection at altered temperature to drive virus evolution
- Determine cellular receptors for VHSV
- Investigate the determinants of virulence for VHSV

11. Characterize Immune Response of Fish to VHSV

This research will investigate the natural immune response of Great Lakes fish to VHSV infection using a laboratory challenge model.

- Develop assays for assessing innate and adaptive immune responses in model fish species
- Develop assays for humoral and cellular immunity for model fish species
- Characterize magnitude and onset of innate immunity to VHSV infection
- Characterize the magnitude and duration of long-term adaptive immunity
- Test the effect of temperature on the onset, strength and duration of the immune response

12. Development and Testing of Candidate Vaccines

One management strategy to protect aquaculture from the effects of the Great Lakes strain of VHSV is vaccination. Prevention of VHSV infection and disease in cultured fish would also benefit wild fish by avoiding amplification of the pathogen in high density cultured populations.

- Test efficacy of autogenous, killed viral vaccines in model species
- Construct a DNA vaccine using G gene of VHSV IVb
- Test efficacy and breadth of protection of DNA vaccine in relevant aquaculture species
- Test magnitude and duration of the immune response to different vaccines
- Test novel methods for mass immunization of fish with killed or DNA vaccines

Killed, attenuated and DNA vaccines have been developed in Europe against VHSV (genotype Ia). These should work with little modification for VHSV IVb; however, killed and DNA vaccines against fish rhabdoviruses require injection (i.p and i.m., respectively) and the attenuated strains have proven difficult to license. With the exception of high-value stocks (brood stock, Atlantic salmon smolts, koi), these vaccines are likely to have limited availability without automated or novel mass delivery methods.

13. Research in Support of Policy Development

- Determine magnitude and directions of baitfish movement
- Test susceptibility and carrier state of important baitfish species at relevant temperatures
- Determine magnitude and directions of ornamental fish movement
- Test susceptibility and carrier state of important ornamental fish species at relevant temperatures
- Investigate the risk of VHSV type IVa from the west coast to fish in other areas

Information about baitfish movement in Ontario, Canada has been collected and shows a higher than anticipated level of movement and discard of live bait. Initial work showed the west coast isolate of VHSV is highly virulent for yellow perch.